AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 108:409–425 (1999)

Investigations Into the Effect of Diet on Modern Human Hair Isotopic Values

T.C. O'CONNELL* AND R.E.M. HEDGES
Research Laboratory for Archaeology and the History of Art, University of Oxford, Oxford, OX1 3QJ, United Kingdom

KEY WORDS carbon; nitrogen; isotopic analysis; animal protein consumption; diet; humans

ABSTRACT Carbon and nitrogen isotopic analysis of body tissues is one of the few techniques that can furnish quantitative information about the diet of archaeological humans.

The study of the effects of various diets on modern human isotopic values can help to refine palaeodietary theories, and such work also enables the testing of palaeodietary theories independent of archaeological remains and interpretations.

This report discusses the use of modern human hair as a sample material for isotopic analysis. The biogenic carbon and nitrogen isotopic signal is well preserved in hair, and the isotopic values of the keratin can be related to diet. We show that atmospheric and cosmetic contamination of hair keratin does not appear to affect the measured isotopic values.

In a small study of Oxford residents, we demonstrate that the magnitude of the nitrogen isotopic values of hair keratin reflects the proportion of animal protein consumed in the diet: omnivores and ovo-lacto-vegetarians have higher $\delta^{15}N$ than vegans. There was an observed relationship between the reported amount of animal protein eaten (either meat or secondary animal products) and the nitrogen isotopic values within the two groups of omnivores and ovo-lacto-vegetarians, indicating that an increasing amount of animal protein in the diet results in an increase in the $\delta^{15}N$ of hair keratin. This provides the first independent support for a long-held theory that, for individuals within a single population, a diet high in meat equates to elevated nitrogen isotopic values in the body relative to others eating less animal protein.

The implications of such results for the magnitude of the trophic level effect are discussed. Results presented here also permit a consideration of the effects of a change of diet in the short and long term on hair keratin isotopic values. Am J Phys Anthropol 108:409–425, 1999.

The ability to make quantitative statements about ancient diets has fundamental importance for archaeology, since diet reflects the interaction between demography, economy, environment, and food-production technology. However, direct archaeological evidence of diet is often elusive. Since "you are what you eat," the biochemical analysis of body tissues is an indirect analysis of the food consumed. The natural distribution of stable isotopes in biological systems enables us to quantify aspects of food uptake into the body, and carbon and nitrogen isotopic analy-

Grant sponsor: SERC/NERC.

*Correspondence to: T.C. O'Connell, 6 Keble Road, Oxford, OX1 3QJ, UK. E-mail: tamsin@ermine.ox.ac.uk

Received 31 October 1997; accepted 16 December 1998.

sis of archaeological human bone is often the only quantitative and objective technique available for the reconstruction of palaeodiet (Schwarcz and Schoeninger, 1991).

PALAEODIETARY ANALYSIS

Early dietary interpretations of archaeological human isotopic data were based on comparison with isotopic values of modern plants and animals (Chisholm et al., 1982, 1983; Tauber, 1981; Vogel, 1978; Vogel and van der Merwe, 1977). The recognition of systematic isotopic patterns in natural food webs led to laboratory-based animal-feeding studies in an attempt to quantify the patterns observed (Ambrose and Norr, 1993; DeNiro and Epstein, 1978, 1981; Katzenberg and Krouse, 1989; Nakagawa et al., 1985; Tieszen and Boutton, 1988; Tieszen et al., 1983; Tieszen and Fagre, 1993). These experiments supplied primary information about the fate of macronutrients in the body's metabolic processes and have provided the basic tenets that underlie the current theories of palaeodietary reconstruction.

Archaeological human diet has been analysed by extrapolating the results of these systematic investigations into the isotopic relationship between diet and body tissues in animals. Although animal studies provide important information about relationships between diet and body tissues, it is of questionable validity to use these results as a basis for the interpretation of specific human dietary situations. Although the basic physiology of humans and animals is similar, the question of size, growth, and efficiency of assimilation of the diet may call into question the possibility of using animals as direct comparisons for humans. In addition, the effect of growth (when the animal is in negative nitrogen balance) on isotopic values has not been quantified, and the majority of animal studies are performed on animals that are either still growing or that have not been maintained in a steady state for a long period of time. The study of the effects of various diets on modern human isotopic values should provide direct information on the detailed isotopic effects of particular dietary variations and can be used to refine and extend palaeodietary theories. Systematic studies of dietary effects on modern humans also permit the independent testing of palaeodietary theories developed via animal studies.

Isotopic analysis of modern human tissues

The effect of dietary variation on the isotopic values of modern (living) human body tissues has rarely been directly studied. Part of the problem has been one of sample material. Archaeological human isotopic analyses are usually of bone collagen, since bones are often the only part of the body recovered after significant burial periods. Most previous animal isotopic studies have also concentrated on bone collagen and the relationship between bone collagen isotopic values and diet. An advantage of using bone collagen for palaeodietary analysis is that it has a long turnover period and as such reflects the diet consumed over a period of about 10 years (Stenhouse and Baxter, 1979), although this figure is debated and may be somewhere in the region of 2–20 years, depending on the age and health of the individual and the type of bone considered (Ambrose, 1993). However, bone is not a readily available sample material from most modern living humans. In most investigations, hair has been used as a representative sample material of all body tissues (Minagawa, 1992; Nakamura et al., 1982; Webb et al., 1980; Yoshinaga et al., 1996), while some have used hair, urine, and blood plasma as indicators of body isotopic values (Katzenberg and Krouse, 1989; Schoeller et al., 1986).

Hair keratin has been used as an alternative modern human body tissue because it is easy to sample and the limited animal data available suggest that, like collagen, keratin isotopic values closely reflect diet. The carbon isotopic values of the hair protein keratin correlate well with diet, being enriched by +1–2‰ relative to dietary protein (DeNiro and Epstein, 1978; Jones et al., 1981; Katzenberg and Krouse, 1989; Tieszen and Fagre, 1993). The nitrogen isotopic values of most body proteins including collagen, keratin, and muscle protein are very similar, and these correlate well with diet, generally enriched by 2–3‰ (Ambrose, 1993; DeNiro

and Epstein, 1981; Hare et al., 1991; Nakagawa et al., 1985; Sealy et al., 1987). Hair appears to be representative of the isotopic composition of human tissues, although it has a much faster turnover time than collagen, which may affect the measured isotopic values. This is discussed in more detail later.

Some of the previous modern hair studies have examined how the variations in an individual's hair keratin isotopic values can be related to the average isotopic values of the typical diet of their geographical location (Katzenberg and Krouse, 1989; Minagawa, 1992; Nakamura et al., 1982; Wada et al., 1991). Other studies have estimated the isotopic enrichment between diet and hair keratin in modern humans (Schoeller et al., 1986; Yoshinaga et al., 1996). In agreement with the available animal isotopic data, these studies of modern human hair suggest that hair isotopic values do reflect the isotopic composition of the diet. However, such work has not quantitatively investigated the effect of dietary variation on the isotopic values in the human body.

Studying modern human diet using isotopic analysis of hair. These previous studies have shown that hair can be used as isotopically representative of human body tissues and suggest that hair from modern humans can be used to study the effects of different dietary composition on the isotopic values of the human body. We have used this approach to investigate one possible effect: that differing levels of meat intake (strictly defined as animal protein) have a measurable influence on human body tissue isotopic values. A decision to eat or not to eat meat and animal products is a common modern dietary choice. Therefore, the isotopic analysis of modern human hair from individuals with varying diets can be used to study the effect of levels of animal protein on the isotopic values of human body tissues.

Levels of animal protein in human diet

Estimation of the amount of meat or animal protein intake in the diet of archaeological populations is important in establishing the type of palaeoeconomy (e.g., an agricultural subsistence as opposed to a pastoral or hunting society). Methods of assessing diet and specifically the importance of animal protein consumption include analysis of faunal remains, tooth microwear, and palaeopathology; however, these techniques are qualitative. Isotopic analysis has been suggested as a possible quantitative method of establishing animal protein consumption in archaeological humans.

It is known that body tissues such as bone collagen and hair keratin are more positive in $\delta^{15}N$ by +3‰ relative to diet (DeNiro and Epstein, 1981; Hare et al., 1991). This results in increasing $\delta^{15}N$ values as the food chain is ascended: carnivores have a higher $\delta^{15}N$ than the herbivores on which they feed, while herbivores have a higher $\delta^{15}N$ than plants (Schoeninger and DeNiro, 1984). The increase in $\delta^{15}N$ up the food chain is termed the trophic level effect; it is a consequence of and is equivalent in magnitude to the enrichment in ^{15}N levels between an individual's diet and its body. From the trophic level effect, it has been argued that a diet low in meat (a low mean dietary $\delta^{15}N$ value) produces low $\delta^{15}N$ values in the body proteins of the consumer and one high in meat (a high mean dietary $\delta^{15}N$ value) produces high $\delta^{15}N$ values in the consumer.

Although this argument has been applied to archaeological humans in an attempt to estimate the animal protein content of their diet, the postulated theory has not yet been demonstrated. It is necessary, in order to avoid circular arguments, to prove a direct relationship between increasing levels of animal protein consumption and human nitrogen isotopic values independent of the archaeological evidence.

Modern human carbon isotopic variation and animal protein consumption. Work by Webb et al. (1980) attempted to assess the specific effects of animal protein intake on hair isotopic values from individuals within the same population; however, they measured only the carbon isotopic values. After the $\delta^{13}C$ of hair keratin from omnivores and ovo-lacto-vegetarians (those individuals eating no animal flesh but consuming secondary animal protein such as milk and eggs) in Australia and New Zealand was measured, there was found to be little effect on $\delta^{13}C$ from the consumption of

secondary animal protein as opposed to meat. In retrospect, the results are not surprising. As Webb et al. (1980) discuss, isotopic values of food in the literature show that all animal-derived protein from the same individual—a chicken and its eggs, a cow's meat and its milk—are isotopically equivalent in both carbon and nitrogen and can all be classed as animal protein for the sake of isotopic dietary reconstruction (Katzenberg and Krouse, 1989; Minagawa, 1992; Schoeller et al., 1986). Consequently, there is no fundamental carbon isotopic difference between the dietary components of omnivores and ovo-lacto-vegetarians and therefore no expected difference between their carbon isotopic values.

Nitrogen isotopic variation. To be able to comment on the dietary composition of archaeological humans from nitrogen isotopic values, we must understand which factors affect these values. As stated previously, the primary source of human nitrogen isotopic variation is diet. Secondary influences such as climate, rainfall, and animal physiology may also play a part (e.g. water stress, pregnancy, and lactation) but have not yet been fully quantified (Ambrose and DeNiro, 1986; Gröcke et al., 1997; Koch, 1997; Sealy et al., 1987).

When the effect of diet on nitrogen isotopic values is considered, there are two sorts of variation: diet composition (marine vs. terrestrial, amount of animal protein, etc.) and also baseline variation in the food chain $\delta^{15}N$. Plant nitrogen isotopic variation is known to vary widely between ecosystems, owing to climate, environmental conditions, and the nitrogen content and isotopic values of soils (Handley et al., 1994; Handley and Raven, 1992; Heaton, 1987). This makes comparisons between archaeological populations difficult unless there are reference plant and animal food source materials available.

Therefore, for nitrogen isotopic values of archaeological human samples to be used to investigate meat or animal protein consumption in archaeological humans, there are two areas that must be investigated. Interecosystem nitrogen isotopic variation must be assessed to investigate the range of variation in nitrogen isotopic values and to determine whether nitrogen isotopic values can be compared between populations. In addition, we need to show whether, within a single population, variations in diet composition result in observable variations in $\delta^{15}N$. In this study we investigate the latter point by examining whether the level of dietary animal protein determines the $\delta^{15}N$ of body tissues. This has not been previously demonstrated.

Breast-feeding and weaning studies. Weaning studies have shown an observable trophic level effect in humans, with breast-feeding infants enriched by about 3‰ in $\delta^{15}N$ relative to their mother (Fogel et al., 1989; Katzenberg and Pfeiffer, 1995; O'Connell and Hedges, unpublished data). Since infants have a high animal protein intake, it might be suggested that this signal demonstrates that a high animal protein intake results in enriched nitrogen isotopic values. But a trophic level signal in humans owing to breast-feeding cannot be compared to variation in adult human $\delta^{15}N$ owing to differences in diet selection and composition.

The $\delta^{15}N$ signal in breast-feeding infants indicates an enrichment in nitrogen isotopic values between diet and body tissues. However, it does not indicate that, given a choice of foods, the amount of animal protein consumed affects the $\delta^{15}N$ of an individual's body tissues. To demonstrate this effect, we must compare the nitrogen isotopic values of body tissues of individuals within a single population who have varying levels of animal protein intake, and, for such a comparison to be valid, the available animal and vegetable protein that may be consumed by each individual must be assumed to have similar $\delta^{15}N$ values. This is not the case with breast-fed infant and mother, since the nitrogen isotopic values of the diets of the infant and mother are not comparable (breast-fed infants are one step directly above their mothers in the food chain). In addition, infants are in a rapid state of growth, and this may affect their isotopic values to some degree. The weaning signal therefore cannot confirm that varying $\delta^{15}N$ values can be seen in adult humans (in a metabolic steady

state) from varying animal protein intake alone.

This study. We propose to test the hypothesis that the level of animal protein in an adult individual's diet produces a detectable and quantifiable signal in the $\delta^{15}N$ of their body tissues. We have compared the isotopic values of human hair from adults in the same population who have varying meat and animal protein consumption.

CONSIDERATIONS IN USING HAIR KERATIN

Before using hair keratin to study the effects of a change in diet composition on the isotopic values of human body tissues, we must consider both the effects of the rate of hair growth and metabolic turnover on hair isotopic values. Unlike most body proteins which are remodelled throughout an animal's lifetime, hair is unusual in that the protein is not reabsorbed. The carbon and nitrogen isotopic values of proteins with a slow turnover rate, such as bone collagen, reflect an average of the body's dietary protein intake over a long period of time (Stenhouse and Baxter, 1979), but the carbon and nitrogen isotopic values of hair along the hair shaft length approximate to a linear record of the most recent diet. With a growth rate of about 1cm each month (Saitoh et al., 1969), a fairly short length of 6 cm covers the diet of the last 6 months. This unique property may used to monitor such parameters as seasonal dietary change. White (1993; White and Schwarcz, 1994) has used $\delta^{13}C$ and $\delta^{15}N$ in hair to determine seasonal change in diet. The change of 2–4‰ that she found in the $\delta^{13}C$ values of Nubian mummy hair can be attributed to a shift in diet from C_3 to C_4 plants. However, it is not known how great a dietary shift is required before the signal shows in the isotopic values of hair or how long the time period is before the change in diet is registered in the isotopic values of hair. If a seasonal diet change is cyclical and we do not know the time period required for humans to reach isotopic equilibrium after a change in diet, then we cannot use such isotopic analyses to quantify by how much the diet has changed.

Physical considerations

Nakamura et al. (1982) showed that an isotopic shift resulting from dietary change caused by a change of geographical location takes at least 6–12 days to show in the $\delta^{13}C$ of adult male beard hair, since it takes a minimum of 6 days for the growing hair to emerge from the skin (Saitoh et al., 1969; Valkovic, 1977). In addition, when the time lag for stable isotope ratios to be reflected in hair is considered, it must be remembered that at any one time individual hair follicles all over the scalp are at different stages in the growth cycle. The hair growth cycle has three time phases: the long anagen, or growing, phase, the short transitional catagen phase, and the intermediate telogen, or resting, phase. In humans, each follicle's growth pattern is independent of the ones around it, so hairs that are adjacent on the scalp and assumed to be contemporaneous can be out of step with each other. Human scalp hair follicles spend on average 3 or more years in the anagen phase, 1–2 weeks in the catagen phase, and 3–4 months in the telogen phase (Valkovic, 1977). Thus, any sample taken will have an average of 88% of hairs growing and 11% static, and therefore approximately 10% of the isotopic signal will be between 0–3 months behind in reflecting the diet.

Metabolic considerations

The initial effects of any isotopic variation within the body caused by a change in diet can be seen within days (Nakamura et al., 1982). However, the transition to an isotopic steady state reflecting the new diet will take a longer period of time. Most proteins in the body are constantly being resorbed and remodelled (excluding hair, fingernails, and skin). Protein synthesis rates in the human body are such that every day the body typically produces three to five times as much protein as the average daily protein intake (Davidson and Passmore, 1979). Thus, approximately one-quarter of the amino acids required for protein regeneration within the body is supplied by the dietary intake, and the rest is taken from the breakdown products (amino acids) of other body proteins, or from the body protein pool. This protein pool acts as an isotopic buffer and

reduces the effect of short-term isotopic fluctuation in the diet. Similarly, after a change in diet isotopic composition, the body proteins formed prior to the dietary change are a reservoir of amino acids that prevent the isotopic values of newly synthesised body tissues from immediately shifting to the isotopic composition of the new diet. Therefore, after a change in diet, the isotopic values of all body proteins change gradually as the protein pool is slowly modified to the isotopic value of the new diet.

It is not known how long the body protein pool in humans takes to isotopically equilibrate after a change in diet. In a study on young steers, Jones et al. (1981) showed that cattle hair took at least 74 days to equilibrate after a shift from a C_4 to C_3 diet. It would be expected that cattle would take a longer time to reach equilibrium than humans, since cattle have a larger protein reservoir within the body which should take longer to equilibrate to the new isotopic composition. However, no previous study has examined the isotopic equilibration time for humans.

MATERIALS AND METHODS

In this study, hair samples were collected and isotopically analysed from 28 individuals with varying diets, and the results correlated with the amount of animal protein they consumed. The effects on hair isotopic values of a change in diet in the short and long term were studied to consider the effects of metabolic turnover on the isotopic signal measured. Prior to these experiments, we considered how best to measure the isotopic signal in the hair sample and what effects atmospheric and cosmetic contamination might have on hair isotopic values. In the experimental section, we detail considerations of isotopic analysis and also experiments performed to investigate the effects of contamination using both animal (previously untreated) and human hair as a test material.

Dietary variation and the effect on hair isotopic values

The isotopic values of hair keratin from individuals with differing diets in the same population were measured. Hair samples were collected from local adult Oxford residents. The samples were taken from each subject in the same way. A small hair sample (15–20 hairs) was cut from the crown of the scalp, the scalp end of the sample wrapped in micropore tape to anchor the strands, and the hair stored in a plastic bag until it was analysed. Analysis was as described in the following methodological section. All subjects gave details of their diet over the year prior to sampling and of their hair care procedure, such as the frequency of washing and any special treatments or dyes used. Diet details taken included listing the types of protein, carbohydrate, fats, and sugars consumed as well as the frequency of consumption.

Twenty-eight individuals gave hair samples, and the subjects were placed into one of three groups: omnivores (numbering 14), ovo-lacto-vegetarians (numbering six) and vegans (numbering eight). Vegans were those consuming no animal produce at all. Others who did not eat meat or marine foods but who did eat secondary animal products such as eggs and dairy produce were placed in the ovo-lacto-vegetarian group. There was a wide range in the reported frequency of consumption of secondary animal products in this group, ranging from daily to rarely. The third group, omnivores, were those that ate meat, secondary animal products, and marine foods. Again there was a wide range of reported frequency of animal protein consumption, from daily to once or twice weekly. None of the subjects had a significant direct consumption of C_4 plants such as maize. None of the omnivores reported more than an infrequent consumption of marine foods. All 28 had not significantly changed their eating habits in the 3 years prior to sampling.

Effect of a change in dietary composition. It was possible to investigate the effects of a change in diet on the isotopic values of hair in both the short term and in the long term. Short term effects were observed by analysing hair from one individual (not included in the main study) who had changed from an omnivorous to a vegan diet 15 months prior to giving a hair sample. The length of hair taken (23 cm) covers the

time period before and after the change in diet. Hair was also sampled from the subject's husband, who had been a vegan for over 5 years. The two had eaten an identical diet over the 15 months prior to the hair being sampled. Long-term effects were observed by analysing hair from one of the current vegan subjects. The individual had kept a length of hair cut off approximately 22 years earlier, when her diet was omnivorous and high in marine foods and meat. The first sample is from 1972, while an omnivore. The individual changed from an omnivorous diet to a vegan diet in 1974, and the second sample is from 1994, while a vegan.

Analytical procedures

Sample cleaning. Unlike most body proteins, hair is entirely exposed to the external environment, so even modern samples are liable to contamination. Cleaning of the hair samples prior to analysis is vital to remove any possible surface contaminants, such as sebum lipids, shampoo residues, or particulate matter. Samples were cleaned by soaking in a 2:1 mixture of methanol and chloroform for about 2 h to remove any lipid or shampoo residue and then rinsed twice in water. All reagents used were of analytical grade or above, and all water used was deionised and distilled. Organic solvents (acetone, methanol, and chloroform) have been used previously in such work (Katzenberg and Krouse, 1989; Minagawa, 1992; Nakamura et al., 1982; Schoeller et al., 1986; Webb et al., 1980; Yoshinaga et al., 1996). Detergents were not used since these have been shown to be damaging to the hair surface (Taylor et al., 1995).

Sample preparation and analysis. Isotopic analyses were performed using an automated carbon and nitrogen analyser and a continuous-flow isotope-ratio-monitoring mass spectrometer (cf-irm-ms) (ANCA Roboprep coupled to a 20/20 mass spectrometer; Europa Crewe, UK). Typical replicate measurement errors are of the order of $\pm 0.3‰$ for $\delta^{13}C$ and $\pm 0.4‰$ for $\delta^{15}N$ (O'Connell, 1996).

Hair samples were wrapped in aluminium foil and cut into sections for loading into the cf-irm-ms. The entire hair sample was placed lengthways onto a long strip of aluminium foil approximately 15 mm wide; then this strip of foil was folded over twice to enclose the hair sample. Problems with static electricity were avoided by keeping the sample wet. The hair wrapped in foil was then sectioned into 1–2 cm lengths and dried overnight under vacuum to remove any remaining water. After drying, the aluminium lengths were rolled into balls and then loaded into the carousel of the cf-irm-ms ready for combustion.

Background signals of carbon and nitrogen from the aluminium foil were negligible (O'Connell, 1996). The cf-irm-ms permitted the analysis of hair samples between 600 and 3,000 µg in mass.

Combustion, contamination, and C/N ratios. The theoretical carbon/nitrogen (C/N) atomic ratio of keratin is 3.4. From the analyses performed, the measured C/N ratios in modern hair samples varied by up to ± 0.5 from the theoretical C/N value of 3.4 (O'Connell, 1996). A range of measured C/N ratios was also seen between different sections of one sample of hair from an individual, usually of the order of 0.25 but up to a maximum value of 0.6 in one individual. However, alongside this intraindividual variation in C/N ratio, there was little concurrent variation in isotopic values. Therefore, it must be concluded that the possible range of C/N ratios in modern hair is between 2.9 and 3.8, and this wide variation is not an artefact of the experimental analysis. Any samples with C/N ratios outside the range of 2.9–3.8 were deemed not to have been satisfactorily combusted and excluded from the data set.

For archaeological collagen isotopic analyses, the C/N ratio can be used to confirm that the sample is not contaminated with organic materials (DeNiro, 1985). Any variation outside the range 2.9–3.6 is taken to indicate diagenetic contamination. The possibility of using the C/N ratios of hair keratin in a similar way was considered. However, due to the wide variation (2.9–3.8) found in the C/N ratio, this cannot be taken as a sensitive indicator of contamination. A sample with a keratin C/N ratio of 3.1 could be contaminated with 20% extraneous carbon (e.g., with lipids that have a high carbon and low

TABLE 1. Effect of various cleaning treatments on horse hair keratin

Treatment	Number of samples	C/N ratio		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
		Mean	Std dev	Mean	Std dev	Mean	Std dev
Uncleaned	2	3.13	0.00	−26.0	0.1	5.5	0.2
Cleaned	3	3.08	0.12	−26.0	0.4	5.6	0.1
Shampooed but uncleaned	8	3.15	0.16	−25.9	0.3	5.3	0.2
Shampooed and cleaned	4	3.23	0.03	−25.7	0.1	4.6	0.2

TABLE 2. Effect of various treatments on hair keratin

Treatment	Number of samples	C/N ratio		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
		Mean	Std dev	Mean	Std dev	Mean	Std dev
Uncleaned	3	3.42	0.03	−20.8	0.2	9.6	0.1
Cleaned	17	3.53	0.08	−20.7	0.4	9.3	0.1
Hennaed	3	3.54	0.06	−20.4	0.2	9.2	0.2
Bleached	5	3.28	0.04	−20.0	0.2	8.8	0.2

nitrogen content) and still have an acceptable C/N ratio of 3.7. In summary, the C/N ratios of all samples should be calculated but can be used only as a very general indicator of a sample's quality.

Experiments addressing contamination of keratin

Exogenous contamination is a problem when considering archaeological samples but is also a concern when using a modern sample material such as hair which is exposed to environmental alteration. Contamination may result from environmental pollution, treatments such as shampoos, and other cosmetic treatments such as dyes. Other factors such as loss of pigment (greying) may also affect the C/N ratios and isotopic values. Brief experiments were carried out to assess how prone hair is to different forms of contamination.

Shampooing and cleaning. The C/N ratios and isotopic values of horse hair (previously untreated in any way) were unaffected by soaking overnight in a protein-based shampoo, by soaking in shampoo and then washing with an organic solvent (methanol and chloroform), or by washing only with a solvent (methanol and chloroform) (Table 1). It is surmised that the effect of shampoo on the stable isotope ratios of hair is too small to be significant in these experiments.

Dyes. Organic colourants such as henna, a plant-derived dye, could elevate the C/N ratios and affect the isotopic values of hair if sufficient quantities were adsorbed onto the hair shaft. Bleaching by hydrogen peroxide (a strong denaturing agent) and other such cosmetic treatments could alter the C/N ratio and isotopic values of hair since such processes are known to damage hair by the removal of amino acids, such as cysteine (Baba et al., 1973). We found that the C/N ratios of human hair were unaffected by dyeing with henna but that bleaching by peroxide effected a small difference in C/N ratios and isotopic values (Table 2). The C/N ratio of hair after bleaching was lower than that of the untreated sample but was still well within the range of 2.9–3.8 found in other untreated samples analysed during this study. Similarly, the C/N ratios of human hair samples from three individuals (not included in the diet study) who repeatedly dyed their hair were also within this range of 2.9–3.8. Therefore, although it is not necessary to automatically exclude dyed or treated samples, care should be taken when isotopically analysing modern hair samples that have been frequently or aggressively treated with denaturing agents and other dyes.

Grey hair. There was no difference between the C/N ratios or the isotopic values of grey and nongrey hair from members of the same family with the same diet (Table 3), implying that lack of pigment in the hair has no observable effect on its isotopic values.

TABLE 3. Variability in the measured C/N ratios of grey and pigmented hair

Subject	Sample length (cm)	Number of sections	C/N ratio	
			Mean	Std dev
Grey 1	12	6	3.48	0.07
Grey 2	12	6	3.36	0.06
Grey 3	8	4	3.39	0.06
Nongrey 1	8	4	3.51	0.08
Nongrey 2	12	6	3.35	0.03

This is in agreement with Minagawa (1992) findings in Japan.

Environmental contamination. There were no statistically significant variations between the isotopic values and C/N ratios of different types of body hair (scalp, axillary, and pubic) from the same individual (Table 4). This is similar to the results of DeAntonio et al. (1982), who found no difference in the chromium concentrations in scalp and pubic hair from the same individual, implying that scalp hair was not preferentially affected by chromium in the atmosphere. It is therefore assumed that atmospheric contamination effects on the isotopic values of modern hair can be discounted.

RESULTS

Different dietary groups

Isotopic analyses of all hair samples collected for dietary analysis were judged to be valid. The C/N ratios of all samples were within the range 3.0–3.7. The standard deviations of the analyses of separate sections within a whole sample from an individual were less than 0.5‰ in $\delta^{13}C$ and 0.4‰ in $\delta^{15}N$, implying that each individual had a relatively constant diet as represented by the length of hair analysed. Results are presented in Tables 5 and 6 and Figures 1 and 2.

Nitrogen data. A comparison of the mean nitrogen isotopic values for all subjects (Table 6; Fig. 2) shows that there is a statistically significant distinction between the nitrogen isotopic values of hair from vegans and from the two other groups (Student's *t*-test: comparing vegans and ovo-lacto-vegetarians, $t = 7.49$, P $t = 9.55$, P 0.0001, 18 degrees of freedom). The hair from vegans is 2‰ lower in $\delta^{15}N$ than that from omnivores or ovo-lacto-vegetarians.

There is no statistical difference in hair $\delta^{15}N$ between omnivores and ovo-lacto-vegetarians (Student's *t*-test, $t = 0.41$, $P = 0.69$, 10 degrees of freedom). As mentioned previously, nitrogen isotopic values of food in the literature show that all animal-derived protein from the same individual, including meat, eggs, and dairy products, are approximately isotopically equivalent and can be classed as animal protein for the sake of isotopic dietary reconstruction (Katzenberg and Krouse, 1989; Minagawa, 1992; Schoeller et al., 1986). Consequently, there is no fundamental difference between the diets of omnivores and ovo-lacto-vegetarians; the diets have components of similar isotopic composition, and the quantity of consumption is the only variable.

When the results are examined in more detail, within the two groups of omnivores and ovo-lacto-vegetarians there is a correlation between an increasing amount of meat or animal protein reported eaten and an increasing $\delta^{15}N$ (Table 7; Fig. 3). Those omnivores or ovo-lacto-vegetarians who reported an infrequent animal protein intake (once or twice weekly) had $\delta^{15}N$ of around 8.4‰, those eating animal protein more often had higher $\delta^{15}N$ values (8.6‰), while those individuals with $\delta^{15}N$ values of over 9.2‰ ate meat and dairy products once daily or more frequently. Ovo-lacto-vegetarians had a maximum $\delta^{15}N$ of 9.4‰, while for omnivores the maximum $\delta^{15}N$ was 9.6‰ (Table 8).

Some of the omnivores in this study recorded that they ate marine foods on average once weekly. Marine foods have a higher $\delta^{15}N$ and $\delta^{13}C$ than terrestrial C_3 foods, and, although the proportion of dietary protein coming directly from marine foods was estimated to be less than 10% for these individuals, consumption of marine foods could have contributed to a slightly higher $\delta^{15}N$ in those individuals. However, the similarity (difference of 0.2‰) in the maximum $\delta^{15}N$ observed in ovo-lacto-vegetarians (eating no marine foods at all) and omnivores (some eating marine foods once a week) suggests that the consumption of marine foods had little observable effect on the $\delta^{15}N$ of this study population.

TABLE 4. Isotopic values of hair from different areas of the body

Hair type	Number of sections	C/N ratio		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
		Mean	Std dev	Mean	Std dev	Mean	Std dev
Scalp	8	3.29	0.10	−19.9	0.4	9.5	0.3
Axillary	3	3.23	0.13	−19.7	0.6	9.6	0.2
Pubic	3	3.33	0.07	−19.9	0.7	9.5	0.1

TABLE 5. Isotopic analyses of all individuals, grouped by dietary preference

Subject type	Number of sample sections[1]	Mean C/N	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
			Mean	Std dev	Mean	Std dev
Vegans						
V1	4	3.20	−22.3	0.3	7.5	0.3
V2	4	3.39	−21.1	0.5	6.9	0.1
V3	4	3.29	−20.8	0.2	7.1	0.2
V4	4	3.12	−20.3	0.1	6.8	0.2
V5	4	3.07	−20.7	0.1	6.3	0.1
V6	4	3.07	−21.1	0.0	7.3	0.3
V7	4	3.17	−19.6	0.1	6.9	0.3
V8	3	3.04	−21.0	0.2	6.4	0.1
Ovo-lacto-vegetarians						
OLV1	4	3.24	−21.3	0.3	8.4	0.1
OLV2	4	3.61	−21.0	0.2	8.4	0.2
OLV3	4	3.21	−20.7	0.2	9.4	0.2
OLV4	4	3.16	−21.1	0.1	9.2	0.2
OLV5	4	3.10	−20.6	0.1	8.6	0.1
OLV6	4	3.14	−21.0	0.1	8.1	0.3
Omnivores						
O1	4	3.11	−21.2	0.4	9.5	0.1
O2	4	3.31	−19.9	0.5	9.6	0.2
O3	4	3.45	−19.8	0.3	8.6	0.1
O4	4	3.28	−20.2	0.5	8.4	0.1
O5	4	3.51	−20.7	0.3	8.7	0.2
O6	4	3.49	−21.2	0.3	8.7	0.1
O7	4	3.39	−21.0	0.4	8.6	0.2
O8	4	3.39	−19.2	0.1	8.0	0.1
O9	4	3.36	−19.7	0.2	8.1	0.1
O10	4	3.27	−19.5	0.4	8.5	0.1
O11	4	3.34	−20.0	0.4	9.4	0.1
O12	4	3.26	−20.6	0.4	8.9	0.2
O13	4	3.06	−19.7	0.3	8.5	0.4
O14	4	3.02	−19.8	0.1	9.6	0.2

[1] Data from only the first four sections of each hair sample closest to the scalp were used to calculate the isotopic values of each individual. Subject V8's values were calculated from three samples, as the hair sample was only 3 cm long.

TABLE 6. Mean isotopic analyses of each dietary preference group

Subject type	Number of subjects	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
		Mean	Std dev	Mean	Std dev
Vegans	8	−20.9	0.8	6.9	0.5
Ovo-lacto-vegetarians	6	−21.0	0.3	8.7	0.5
Omnivores	14	−20.2	0.7	8.8	0.6

Carbon data. Given the mean $\delta^{13}C$ of each of the three groups, there was no statistically significant shift in hair $\delta^{13}C$ values between vegans and ovo-lacto-vegetarians (Student's *t*-test, $t = 0.29$, $P = 0.78$, 9 degrees of freedom) or vegans and omnivores (Student's *t*-test, $t = 2.13$, $P = 0.06$, 12 degrees of freedom). However, omnivores and ovo-lacto-vegetarians appear to be statistically slightly different (Student's *t*-test, $t = 3.79$, $P = 0.002$, 17 degrees of freedom). In comparison, Webb et al.'s (1980) work found that it was not possible to differentiate between ovo-lacto-vegetarians and omnivores using $\delta^{13}C$ values alone. There was also no correlation between the amount of

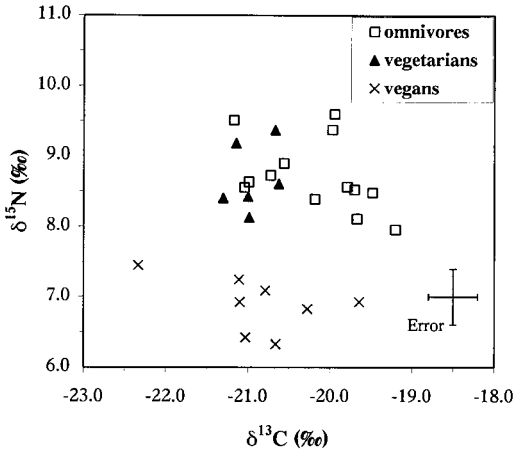

Fig. 1. Mean isotopic analyses from the hair samples of each individual. The mean was calculated using only the first four sections of each hair sample.

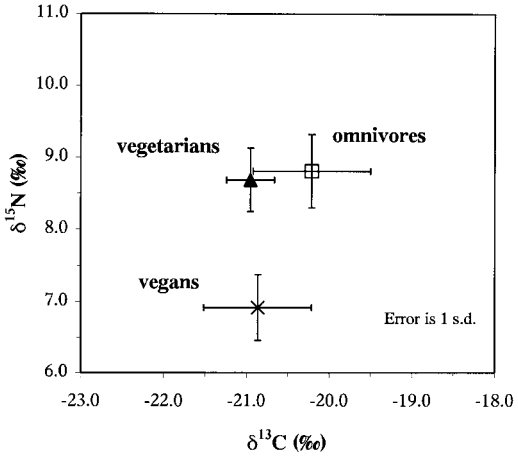

Fig. 2. Mean isotopic analyses for each dietary preference group.

meat or animal protein reported eaten and $\delta^{13}C$ values.

These results indicate that there is little difference in the plant base of the three types of diets and suggests that it is not possible to differentiate the levels of dietary animal protein in this population from $\delta^{13}C$. A possible reason for the small enrichment of omnivores relative to ovo-lacto-vegetarians could have been the consumption of marine foods, but a consideration of the $\delta^{15}N$ (as discussed in the previous section) showed that this was not likely.

TABLE 7. Levels of animal protein consumption and the nitrogen isotopic values of an individual

Subject	Frequency of consumption[1]	$\delta^{15}N$ (‰)
Ovo-lacto-vegetarians		
OLV6	Intermediate	8.1
OLV1	Intermediate	8.4
OLV2	Intermediate	8.4
OLV5	Frequent	8.6
OLV4	Frequent	9.2
OLV3	Daily	9.4
Omnivores		
O8	Intermediate	8.0
O9	Intermediate	8.1
O4	Intermediate	8.4
O10	Intermediate	8.5
O13	Intermediate	8.5
O3	Frequent	8.6
O6	Frequent	8.7
O7	Frequent	8.6
O5	Frequent	8.7
O12	Frequent	8.9
O11	Daily	9.4
O1	Daily	9.5
O2	Daily	9.6
O14	Daily	9.6

[1] Frequency of consumption defined as daily (once or more a day), frequent (more than twice a week), intermediate (once or twice weekly), or rarely (less than once a week).

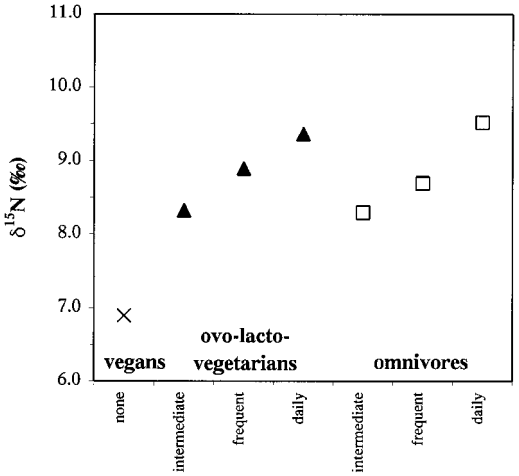

Fig. 3. Dependence of mean hair nitrogen isotopic values on the frequency of animal protein consumption for each dietary group.

The carbon isotopic values of all body proteins are related to the carbon isotopic values of the dietary protein, and it has been shown that hair keratin is enriched relative to diet by +2–3‰ in $\delta^{13}C$ values. When the carbon isotopic values of all subjects are taken as a single sample population, the range observed of -22.6 to -19.0‰ in the

TABLE 8. Variation in mean nitrogen isotopic values with the frequency of animal protein consumption for each dietary group

Dietary group	Frequency of consumption of animal protein	$\delta^{15}N$ (‰)
Vegans	Never	6.9
Ovo-lacto-vegetarians	Intermediate	8.3
	Frequent	8.9
	Daily	9.4
Omnivores	Intermediate	8.3
	Frequent	8.7
	Daily	9.5

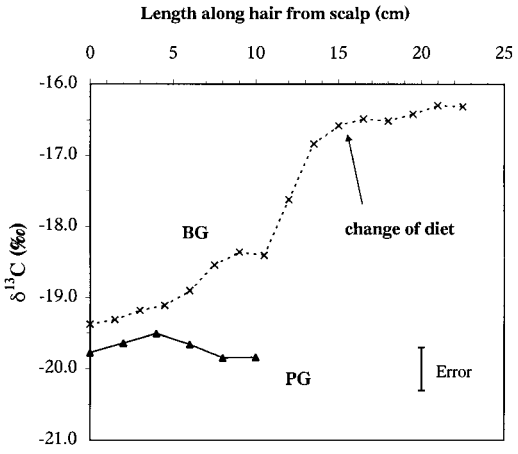

Fig. 4. Effect of a short-term change in diet on the carbon isotopic values of hair keratin.

subjects' hair keratin is typical of a primarily C_3 ecosystem, such as northwest Europe. Most of the subjects recorded that they ate some C_4 plant-derived products such as sweet corn, corn oil, and cane sugar, although such foods were not major dietary components. After we correct for the shift between keratin and collagen $\delta^{13}C$ (approximately +2‰) and the industrial revolution "fossil fuel effect" (modern $\delta^{13}C$ values are -1‰ relative to pre-1800 $\delta^{13}C$ values [DeNiro, 1987]), the range measured in modern human $\delta^{13}C$ is slightly enriched ($\approx$ +2‰) relative to that observed in several inland British prehistoric and Iron Age (farming) populations of about -21 to -19‰ in bone collagen (Richards and van Klinken, 1997; Richards et al., 1998). This enrichment relative to a population that would not have had access to C_4 plants suggests that C_4 foods do supply some proportion of the dietary protein of the individuals studied here.

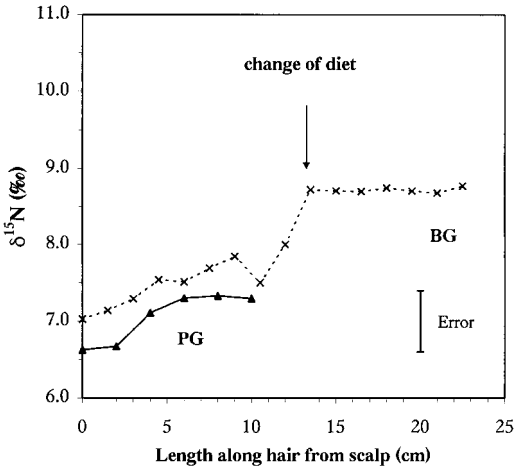

Fig. 5. Effect of a short-term change in diet on the nitrogen isotopic values of hair keratin.

Effects of a change in diet

Short-term change in diet. In the study of short-term change in diet, we observed a dramatic effect on the isotopic values of an individual's hair following a change from an omnivorous to a vegan diet 15 months prior to sampling. The data presented are that of BG, a woman who changed from an omnivorous to a vegan diet (not included in the main study) and of her husband, PG, a vegan for 8 years (Figs. 4, 5). The change in diet for BG also roughly coincided with a move from Texas, USA, to Oxford, UK—hence the enriched initial $\delta^{13}C$ values of her hair samples (USA residents are more enriched in $\delta^{13}C$ than northwest Europeans (Schoeller et al., 1986) because of the increased consumption of C_4 plants, both directly and as animal feed; see later discussion). In the first couple of months after the dietary change (1cm of hair length approximates to 1 month of growth), both carbon and nitrogen isotopic values became more depleted (lines marked on the graphs in Figs. 4 and 5 as BG). After 5 months, the nitrogen isotopic values were approaching equilibrium, while the carbon isotopic values had changed two-thirds of the way towards the isotopic values expected from the new diet (as seen in the subject's husband's hair, marked PG in Figs. 4, 5). This initial period of change is followed by a more gradual but steady shift in $\delta^{13}C$ towards the

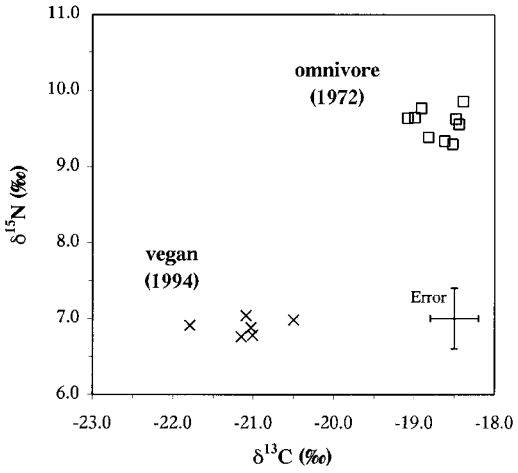

Fig. 6. Effect of a long-term change in diet on the isotopic values of hair keratin.

carbon isotopic values of PG's hair. After 7–12 months, both carbon and nitrogen isotopic values are close to equilibrium. It is interesting that after a year the values of the husband and wife on the same diet for 12 months still have not converged. This may be a result of BG still not having reached equilibrium, or it may reflect small individual preferences in diet; it is not possible to say which is true from the limited dietary information recorded by both subjects.

Long-term change in diet. The long-term effect of a change from an omnivorous to a vegan diet on the isotopic values of hair from individual AB gave similar results to those produced from the isotopic analysis of the different dietary groups (Fig. 6). A comparison of nitrogen isotopic values from the two samples shows a difference of 2.5‰ between hair grown when an omnivore (1972, aged 20–22) and that grown whilst a vegan (1994, aged 44). Since there is no evidence so far to suggest a dependence of isotopic values of the body tissues of a healthy adult on age (Katzenberg et al., 1993; Lovell et al., 1986; White and Schwarcz, 1989), this decrease in $\delta^{15}N$ in the samples from one individual can be ascribed to a decrease in meat or animal protein consumption within that one individual's diet. The change of 2.5‰ is greater than the mean difference between omnivores and vegans in this study (2‰); however, after 21 years the individual AB was unable to assess adequately the retrospective composition of her diet; it is possible that marine foods were a significant proportion of her diet, which would result in elevated $\delta^{15}N$ relative to omnivores who did not consume much marine fish. The striking change in carbon isotopic values is less simple to explain than the nitrogen isotopic values. However, the change is consistent with a change from a diet high in meat and particularly marine foods (both with elevated $\delta^{13}C$ values) to a vegan C_3-based diet.

DISCUSSION

Diet and hair $\delta^{15}N$

This study has showed that hair nitrogen isotopic values can be related to certain aspects of the individual's diet, specifically the amount of animal protein consumed. Taking each dietary group as a whole, we see that vegans have lower nitrogen isotopic values, by 2‰, than omnivores and ovo-lacto-vegetarians, whereas the nitrogen isotopic values of omnivores and ovo-lacto-vegetarians are the same. This similarity between omnivores and ovo-lacto-vegetarians reflects the observation that all animal-derived protein from the same source (meat, milk, eggs) has similar isotopic values. At a more detailed level, there is a systematic relationship between an increasing frequency of animal protein intake and an increase in $\delta^{15}N$ of the individual (Fig. 3).

Diet and hair $\delta^{13}C$

We found no correlation between hair carbon isotopic values and aspects of diet: although ovo-lacto-vegetarians and omnivores were statistically different in $\delta^{13}C$, there was no significant difference between vegans and the other two groups in $\delta^{13}C$.

The modern British humans measured here are more enriched in $\delta^{13}C$ than their prehistoric counterparts, which, given the availability and ubiquity of C_4 plants in the modern world, is unsurprising. However, it might have been expected that omnivores and ovo-lacto-vegetarians might have substantially different carbon isotopic values than vegans. In addition to the direct con-

sumption of C_4 plants, the diet of omnivores and ovo-lacto-vegetarians can contain a hidden indirect C_4 signal, in that maize (corn)-fed chickens and their eggs, together with meat and dairy products from herds eating C_4-derived feed, will have enriched $\delta^{13}C$ values that will be transferred up the food chain, enriching the isotopic signal of the consumers. However, as there was no difference in $\delta^{13}C$ between vegans and ovo-lacto-vegetarians/omnivores, we must infer that either the hidden signal of C_4 animal feed in the diet of omnivores and ovo-lacto-vegetarians is balanced by a concurrent increase of C_4 plant protein consumption by vegans or that all three groups ate a similar proportion of C_4 plant protein; it is not possible to say which.

Effects of a change in diet composition

The shifts in isotopic values of hair in the individual BG immediately following her change from an omnivorous to a vegan diet allows us to examine the equilibration time of the body to a diet of a different isotopic composition (Figs. 4, 5). The pattern seen in the isotopic values of the individual BG following a change in diet is consistent with the initial fast turnover of the majority of the tissues in the body protein pool followed by the slow turnover of other proteins.

Within the first 5 months after the change of diet, most of the body tissue proteins (such as kidney, liver, and muscle) have been broken down and reabsorbed (Tieszen et al., 1983; Waterlow et al., 1978). The rapid change in the isotopic values of the hair reflects this fast turnover of amino acids. After this initial period, the hair keratin is mainly composed of amino acids consumed after the diet change.

However, 5–12 months after the change in diet, the continued slow modification of the hair keratin $\delta^{13}C$ indicates that there were some amino acids being reabsorbed into the keratin that had been released from proteins synthesised prior to the diet change. This indicates the continuing breakdown of proteins such as bone collagen. These results suggest that, for this individual, the body takes at least 7–12 months to equilibrate isotopically with a new diet.

It appears that the $\delta^{13}C$ of the body takes longer to equilibrate after a change in diet than the $\delta^{15}N$ values. This may be a result of the separate and different ways that carbon and nitrogen from amino acids are processed in the body. De- and trans-amination may result in amino groups equilibrating faster than the carbon skeletons of the amino acids; this differing turnover time has not previously been studied.

The measurement of the isotopic values of the two samples of the individual AB's hair marks the first time that changes in the isotopic values of adult human body tissues have been observed over a long period of time following a change in diet (Fig. 6). The analyses show that 20 years after the dietary change the body is in complete isotopic equilibrium with the new diet.

Variations in $\delta^{15}N$ within one trophic level

The use of nitrogen isotopic values to distinguish varying levels of animal protein in the diet is an attempt to investigate small-scale isotopic variation. Given the wide range of observed $\delta^{15}N$ in plants and animals, the accuracy with which the $\delta^{15}N$ of human body tissues can reflect such small differences must be considered.

Nitrogen isotopic values within and between ecosystems are know to vary widely; terrestrial, marine, and freshwater plants can have $\delta^{15}N$ from -2 to +10‰ (Katzenberg and Krouse, 1989; Minagawa, 1992; Schoeninger and DeNiro, 1984; Yoshinaga et al., 1991). One trophic level is taken to be approximately 3‰; consequently, for the assessment of levels of animal protein in the diet from complete herbivory to complete carnivory, there must be fine yet observable gradations within a range of approximately 3‰.

In this study, the individuals were selected at random, and all ate widely differing diets. Yet the change of $\delta^{15}N$ between those with no animal protein and those with a high animal protein intake is within a range of only 2.5‰, and the $\delta^{15}N$ variation within each group was much less (Table 5; the standard deviation of the omnivore group was 0.6‰, and for ovo-lacto-vegetarians and vegans it was 0.5‰). This suggests that, despite the extensive variety of foods avail-

able to north-west Europeans, the average diet for most individuals within each dietary group is isotopically very similar.

In addition, the $\delta^{15}N$ variation along the hair length for each individual was small, with a maximum standard deviation of 0.4‰. Such small variation must be ascribed to the effect of the body protein pool acting as an isotopic buffer, as discussed previously. As a result, despite small possible isotopic fluctuations in diet, the isotopic values of body tissues of each individual are representative of the average of their long-term dietary intake.

We conclude that the isotopic values of body tissues can reflect small mean isotopic differences ($\delta^{15}N$ changes of 1–3‰) in the diet, even in those tissues that have a relatively fast growth rate or turnover time, such as hair.

Magnitude of the trophic level effect

This study demonstrates that the hypothesis outlined earlier in the paper is correct: higher animal protein consumption does result in higher $\delta^{15}N$ values in the body of the consumer. We can use these results to consider the magnitude of the trophic level effect by expressing the hypothesis in the form of the following equations.

First, there is an enrichment in $\delta^{15}N$ between the diet protein and body tissues:

$$\delta^{15}N_{body} = \delta^{15}N_{diet\ protein} + \Delta N, \qquad (1)$$

where $\Delta N = \Delta^{15}N_{(body - diet\ protein)}$

Secondly, that $\delta^{15}N_{diet\ protein}$ is dependent on the proportion of animal protein in the diet:

$$\delta^{15}N_{diet\ protein} = (AP \times M) + ([1 - AP] \times V),$$

where M is the mean $\delta^{15}N$ of animal protein consumed, V is the mean $\delta^{15}N$ of plant protein consumed, and AP is the proportion of dietary nitrogen from animal protein ($0 < AP < 1$) (i.e., $\delta^{15}N_{diet\ protein}$ corresponds to the weighted average of the two sources of dietary protein). This equation can be rearranged to give

$$\delta^{15}N_{diet\ protein} = V + AP \times (M - V). \qquad (2)$$

Now we can consider the magnitude of the trophic level effect as long as we assume that the enrichment of body tissues relative to diet (ΔN) is a constant factor, that $\delta^{15}N_{body}$ is a linear function of the proportion of animal protein in the diet, and that the mean nitrogen isotopic values of the plant and animal proteins consumed are the same for each individual.

Combining equations 1 and 2, we see that

$$\delta^{15}N_{body} = V + AP \times (M - V) + \Delta N, \qquad (3)$$

indicating that the nitrogen isotopic values of the body tissues are composed of contributions from the $\delta^{15}N$ of dietary plant and animal protein (V and M), dependent on the proportions in which they were consumed, together with an enrichment factor, ΔN, that results from the incorporation of dietary components into the body tissues.

Omnivores resident in the United Kingdom typically consume 63% of their dietary protein as animal protein (Davidson and Passmore, 1979). If we make the assumption that the mean isotopic values of all omnivores analysed in this study approximate to the isotopic values expected from a typical omnivore in the United Kingdom, then, from the results of this study, the $\delta^{15}N_{body}$ of vegans (where AP = 0) is 6.9‰, and the $\delta^{15}N_{body}$ of omnivores (where AP = 0.63) is 8.8‰ (Table 6). Using these values in equation 3, for vegans $6.9 = V + \Delta N$, and for omnivores $8.8 = V + 0.63\ (M - V) + \Delta N$. Therefore, $M - V = 3.0$‰. Therefore, the difference between the mean $\delta^{15}N$ values of the animal dietary protein and plant dietary protein is an estimated +3.0‰. Substituting this value back into equation 3, we can evaluate a difference of 3‰ between the body tissues of an individual consuming no animal protein (vegan or complete herbivore) and those of an individual consuming 100% of his or her dietary protein as animal protein (complete carnivore).

The transition up the food chain from herbivore to carnivore is equivalent to one trophic level. Therefore, this estimate of +3‰ is a measure of the isotopic shift between one trophic level and the next, or the trophic level effect. The value derived here is equal to the observed enrichment between trophic levels in a variety of ecosystems (Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984).

CONCLUSIONS

We conclude that in living humans a diet high in animal protein correlates to relatively higher nitrogen isotopic values in hair and one low in animal protein correlates to relatively lower nitrogen isotopic values in hair, demonstrating that the $\delta^{15}N$ values of human body tissues, like those of other mammals, are dependent on the dietary intake of meat or animal protein. We found no such dependence in the carbon isotopic values.

Hair isotopic values reflect diet isotopic values, and if the diet is changed the isotopic values of hair will also change to reflect the new diet. Human hair keratin takes at least 7–12 months to approach equilibrium after a dietary isotopic change.

The expected difference in the nitrogen isotopic values of an individual eating no animal protein (herbivore) and one eating 100% of his or her dietary protein as animal protein (carnivore) is approximately 3‰, which can be equated to one trophic level.

ACKNOWLEDGMENTS

This study would not have been possible without the willing participation of many volunteers; we thank them all. This work was supported by a SERC/NERC studentship for T.C.O'C.

LITERATURE CITED

Ambrose SH. 1993. Isotopic analysis of palaeodiets: methodological and interpretive considerations. In: Sandford MK, editor. Investigations of ancient human tissue. Langhorne, PA: Gordon & Breach Science Publishers. p 59–130.

Ambrose SH, DeNiro MJ. 1986. The isotopic ecology of East African mammals. Oecologia 69:395–406.

Ambrose SH, Norr L. 1993. Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In: Lambert JB, Grupe G, editors. Prehistoric human bone: archaeology at the molecular level. Berlin: Springer Verlag. p 1–37.

Baba N, Nakayama Y, Nozaki F, Tamura T. 1973. Denaturation and release of amino acids in hairs treated with cold-waving lotions, hair-dyes, hair-bleaching solutions and depilatories. J Hyg Chem 19:47.

Chisholm BS, Nelson DE, Schwarcz HP. 1982. Stable-carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science 216:1131–1132.

Chisholm BS, Nelson DE, Schwarcz HP. 1983. Dietary information from $\delta^{13}C$ and $\delta^{15}N$ measurements on bone collagen. PACT 8:391–397.

Davidson S, Passmore R. 1979. Human nutrition and dietetics. Edinburgh: Churchill Livingstone.

DeAntonio SM, Katz SA, Scheiner DM, Wood JD. 1982. Anatomically related variations in trace-metal concentrations in hair. Clin Chem 28:2411–2413.

DeNiro MJ. 1985. Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. Nature 317:806–809.

DeNiro MJ. 1987. Stable isotopy and archaeology. Am Sci 75:182–191.

DeNiro MJ, Epstein S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506.

DeNiro MJ, Epstein S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351.

Fogel ML, Tuross N, Owsley D. 1989. Nitrogen isotope tracers of human lactation in modern and archaeological populations: Annual report of the Director: Geophysical Laboratory, 1988–1989. Washington, DC: Carnegie Institution of Washington. p111–117.

Gröcke DR, Bocherens H, Mariotti A. 1997. Annual rainfall and nitrogen-isotope correlation in macropod collagen: application as a palaeoprecipitation indicator. Earth Planet Sci Lett 153:279–285.

Handley LL, Raven JA. 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant Cell Environ 15:965–985.

Handley LL, Odee D, Scrimgeour CM. 1994. $\delta^{15}N$ and $\delta^{13}C$ patterns in savanna vegetation: dependence on water availability and disturbance. Funct Ecol 8:306–314.

Hare PE, Fogel ML, Stafford TW Jr, Mitchell AD, Hoering TC. 1991. The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. J Archaeol Sci 18:277–292.

Heaton THE. 1987. The $^{15}N/^{14}N$ ratio of plants in South Africa and Namibia: relationship to climate and coastal/saline environments. Oecologia 74:236–246.

Jones RJ, Ludlow MM, Troughton JH, Blunt CG. 1981. Changes in natural carbon isotope ratio of the hair from steers fed diets of C_4, C_3, C_4 species in sequence. Search 12:85–87.

Katzenberg MA, Krouse HR. 1989. Application of stable isotope variation in human tissue to problems in identification. Can Soc Forens Sci J 22:7–19.

Katzenberg MA, Pfeiffer S. 1995. Nitrogen isotope evidence for weaning age in a nineteenth century Canadian skeletal sample. In: Grauer AL, editor. Bodies of evidence: reconstructing history through skeletal analysis. New York: Wiley-Liss. p 221–235.

Katzenberg MA, Saunders SR, Fitzgerald WR. 1993. Age differences in stable carbon and nitrogen isotope ratios in a population of prehistoric maize horticulturalists. Am J Phys Anthropol 90:267–281.

Koch P. 1997. Reproductive status, nitrogen balance, and the isotopic ecology of horses. Presented at the 5th Advanced Seminar on Palaeodiet, France.

Lovell NC, Nelson DE, Schwarcz HP. 1986. Carbon isotope ratios in palaeodiet: lack of age or sex effect. Archaeometry 28:51–55.

Minagawa M. 1992. Reconstruction of human diet from $\delta^{13}C$ and $\delta^{15}N$ in contemporary Japanese hair: a stochastic method for estimating multi-source contribution by double isotopic tracers. Appl Geochem 7:145–158.

Minagawa M, Wada E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta 48:1135–1140.

Nakagawa A, Kitagawa A, Asami M, Nakamura K, Schoeller DA, Slater R, Minagawa M, Kaplan IR. 1985. Evaluation of isotope ratio (IR) mass spectrom-

etry for the study of drug metabolism. Biomed Mass Spec 12:502–506.
Nakamura K, Schoeller DA, Winkler FJ, Schmidt H-L. 1982. Geographical variations in the carbon isotope content of the diet and hair of contemporary man. Biomed Mass Spec 9:390–394.
O'Connell TC. 1996. The isotopic relationship between diet and body proteins: implications for the study of diet in archaeology. DPhil thesis, University of Oxford, UK.
Richards MP, van Klinken GJ. 1997. A survey of European human bone stable carbon and nitrogen values. In: Sinclair AGM, Slater EA, Gowlett JAJ, editors. Archaeological sciences 1995. Oxford: Oxbow Monograph. p 363–368.
Richards MP, Molleson TI, Vogel JC, Hedges REM. 1998. Stable isotope analysis reveals variations in human diet at the Poundbury Camp cemetery site. J Archaeol Sci 25:1247–1252.
Saitoh M, Uzuka M, Sakamoto M, Kobori T. 1969. Rate of hair growth. In: Montagna W, Dobson RL, editors. Hair growth. Oxford: Pergamon Press. p 183–201.
Schoeller DA, Minagawa M, Slater R, Kaplan IR. 1986. Stable isotopes of carbon, nitrogen and hydrogen in the contemporary North American human food web. Ecol Food Nutr 18:159–170.
Schoeninger MJ, DeNiro MJ. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim Cosmochim Acta 48:625–639.
Schwarcz HP, Schoeninger MJ. 1991. Stable isotopic analyses in human nutritional ecology. Yb Phys Anthropol 34:283–321.
Sealy JC, van der Merwe NJ, Lee-Thorp JA, Lanham JL. 1987. Nitrogen isotopic ecology in Southern Africa: implications for environmental and dietary tracing. Geochim Cosmochim Acta 51:2707–2717.
Stenhouse MJ, Baxter MS. 1979. The uptake of bomb ^{14}C in humans. In: Berger R, Suess HE, editors. Radiocarbon dating. Berkeley: University of California Press. p 324–341.
Tauber H. 1981. ^{13}C evidence for dietary habits of prehistoric man in Denmark. Nature 292:332–333.
Taylor RE, Hare PE, Prior C, Kirner D, Wan L, Burky R. 1995. Radiocarbon dating of biochemically characterized hair. Radiocarbon 37:319–330.
Tieszen LL, Boutton TW. 1988. Stable carbon isotopes in terrestrial ecosystem research. In: Rundel PW, Ehleringer JR, Nagy KA, editors. Stable isotopes in ecological research. Berlin and New York: Springer-Verlag. p 167–195.
Tieszen LL, Fagre T. 1993. Effect of diet quality and composition on the isotopic composition of respiratory CO_2, bone collagen, bioapatite, and soft tissues. In: Lambert JB, Grupe G, editors. Prehistoric human bone: archaeology at the molecular level. Berlin: Springer-Verlag. p 121–155.
Tieszen LL, Boutton TW, Tesdahl KG, Slade NA. 1983. Fractionation and turnover of stable isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37.
Valkovic V. 1977. Trace elements in human hair. Amsterdam, NY: Garland Publ. Inc.
Vogel JC. 1978. Isotopic assessment of the dietary habits of ungulates. S Afr J Sci 74:298–301.
Vogel JC, van der Merwe NJ. 1977. Isotopic evidence for early maize cultivation in New York state. Am Antiq 42:238–242.
Wada E, Mizutani H, Minagawa M. 1991. The use of stable isotopes for food web analysis. Crit Rev Food Sci Nutr 30:361–371.
Waterlow JC, Garlick PJ, Millward DJ. 1978. Protein turnover in mammalian tissues and in the whole body. North Holland.
Webb Y, Minson DJ, Dye EA. 1980. A dietary factor influencing ^{13}C content of human hair. Search 11:200–201.
White CD. 1993. Isotopic determination of seasonality in diet and death from Nubian mummy hair. J Archaeol Sci 20:657–666.
White CD, Schwarcz HP. 1989. Ancient Maya diet: as inferred from isotopic and elemental analysis of human bone. J Archaeol Sci 16:451–474.
White CD, Schwarcz HP. 1994. Temporal trends in stable isotopes for Nubian mummy tissues. Am J Phys Anthropol 93:165–187.
Yoshinaga J, Minagwa M, Suzuki T, Ohtsuka R, Kawabe T, Hongo T, Inaoka T, Akimichi T. 1991. Carbon and nitrogen isotopic characterization for Papua New Guinea foods. Ecol Food Nutr 26:17–25.
Yoshinaga J, Minagawa M, Suzuki T, Ohtsuka R, Kawabe T, Inaoka T, Akimichi T. 1996. Stable carbon and nitrogen isotopic composition of diet and hair of Gidran-speaking Papuans. Am J Phys Anthropol 100: 23–34.